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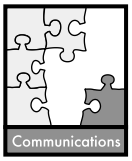
Eising, Corine M.; Robles, Raquel; Lasthuizen, Maarten; Groothuis, Ton G. G.

Published in:
Journal of Avian Biology

DOI:
10.1111/j.1600-048X.2009.04556.x

Publication date:
2009



J. Avian Biol. 40: 635–639, 2009
doi: 10.1111/j.1600-048X.2009.04556.x

Received 21 April 2008, accepted 28 January 2009

Lesser double-collared sunbirds *Nectarinia chalybea* do not compensate for hatching asynchrony by adjusting egg mass or yolk androgens

Corine M. Eising, Raquel Robles, Maarten Lasthuizen and Ton G. G. Groothuis

C. M. Eising (correspondence) and R. Robles, DST/NRF Ctr. of Excellence at the Percy FitzPatrick Inst., Univ. of Cape Town, Rondebosch 7701, South Africa. E-mail: corine.eising@gmail.com. - M. Lasthuizen and T. G. G. Groothuis, Behav. Biol., Univ. of Groningen, 9750 AA, Haren, The Netherlands.

Substantial amounts of maternal androgens are found in birds' eggs and have been shown to benefit offspring development. Within-clutch patterns of increasing androgen concentrations over the laying sequence are often hypothesized to compensate for the negative effects of hatching asynchrony. However, detrimental effects to offspring fitness of exposure to high yolk androgen levels have also been demonstrated. This suggests that mothers should forego these costs to their offspring when the need for compensation for hatching asynchrony is low or when alternative compensatory strategies, e.g. in terms of increasing egg mass, are available. Here we show that in the south-temperate lesser double-collared sunbird *Nectarinia chalybea*, a species with hatching asynchrony but also with high survival of last-hatched chicks, mothers do not deposit resources differentially in terms of either yolk androgen concentration or egg mass across the laying sequence. We discuss to what extend this challenges the original explanation of within-clutch variation in these egg parameters and offer some explanation for their between-clutch variation which was related to female body mass.

Initiation of incubation before clutch completion occurs in many bird species, often resulting in asynchronous hatching. Hatching asynchrony (HA) in turn leads to a competitive hierarchy within the brood, frequently with negative consequences for later-hatched chicks (e.g. see Starck and Ricklefs 1998). Many hypotheses have been put forward to explain the adaptive benefits of HA (e.g. Clark and Wilson 1981, Stenning 1996). These include the 'brood-reduction' hypothesis (Lack 1947, Forbes et al. 1997), the 'egg-viability' hypothesis (Veiga 1992, Cook et al. 2003) and the 'nest-failure' hypothesis (Richter 1982). The first suggests hatching asynchrony facilitates brood reduction when food availability is unpredictable and may be insufficient to raise all chicks. The other two hypotheses suggest that females have no choice but to accept the costs of HA, i.e. reduced survival of last-hatched chicks, because delayed onset of incubation would compromise reproductive output because of reduced hatchability or increased predation risk.

Recent studies show that there are several ways in which mothers can compensate for the detrimental effects of HA, supporting a brood survival strategy. Maternal yolk androgens have been found in the eggs of many species and often increase with the position of the egg in the laying order. Schwabl (1993) was the first to propose that such differential allocation modifies the chick size hierarchy that arises as a consequence of HA thus mitigating its detrimental effects. Subsequently, several other studies of maternal yolk androgen levels, which are listed below, have corroborated this interpretation. Experimental elevation of yolk androgens shortens hatching time (black headed gull *Larus ridibundus*; Eising et al. 2001, 2003; but see Sockman and Schwabl 2000; American kestrel *Falco sparverius*), and comparative studies show a positive relationship between yolk androgen levels and embryonic development rates in passerines (multiple passerine species: Gorman and Williams 2005, Schwabl et al. 2007; the same trend in Gil et al. 2007 did not reach statistical significance). Yolk androgens have also been shown to affect begging behaviour positively (canaries *Serinus canaria*; Schwabl 1993, black headed gulls; Eising and Groothuis 2003, yellow legged gull *Larus michahellis*; Boncoraglio 2006). This is consistent with the finding that yolk androgens stimulate the development of the neck muscle, involved in both hatching and begging behaviour (European starlings *Sturnus vulgaris*; Lipar 2001). However, the few studies that have experimentally manipulated yolk androgens in the context of hatching asynchrony found only slight increases in growth rates of later-hatched chicks (black headed gulls; Eising et al. 2001, canaries; Schwabl 1993), and a concomitantly weak (Eising et al. 2001, European starlings: Pilz et al. 2004), or detrimental (American kestrels: Sockman and Schwabl 2000, reviewed in Groothuis et al. 2005a) effect on chick survival. Therefore, the available results do not consistently support the

hypothesis that maternal yolk androgens compensate detrimental effects of hatching asynchrony.

Within-species comparisons of HA and within-clutch yolk androgen variation yielded contradictory results. Müller et al. (2004) found for black headed gulls that the within clutch increase of yolk testosterone correlated positively with the degree of hatching asynchrony. By contrast, Ellis et al. (house wren *Troglodytes aedon*: 2001) found no differences in within-clutch androgen patterns between synchronously and asynchronously hatching clutches. Clearly, the widely cited hypothesis that avian mothers may compensate for the negative effects of hatching asynchrony by within-clutch variation of androgens in their eggs is in need of further testing and scrutiny.

We investigated whether female lesser double-collared sunbirds *Nectarinia chalybea* compensate for the disadvantages of last-hatched offspring by providing them with increased concentrations of yolk androgens. In this species, incubation starts after the first egg is laid, resulting in asynchronous hatching over one to two days. If not depredated, most chicks that hatch fledge successfully (Schmidt 1964, Skead 1967, Eising pers. obs.), suggesting at least a partial compensatory measure for HA. If this compensation comes in the form of yolk androgens, we expected to find an increase of yolk androgen concentrations over the laying order.

In addition, we also investigated whether there was a pattern of increasing egg mass across the laying order. Beneficial effects of differential egg nutrient allocation for brood survival have been reported (e.g. Hargitai et al. 2005, Rosivall et al. 2005), but simultaneous increases in both egg mass and androgen level have not been documented. Although it remains unclear why the one or other compensatory strategy is more commonly found within species, this could be related to the trade-off between nutrient availability (i.e. costs of egg production) and the costs associated with exposure to high androgen levels to immune function (Groothuis et al. 2005b, Müller et al. 2005). When food availability is not constraining egg size, mothers could be expected to invest more in egg mass, which is costly only to them, rather than in increased yolk hormones, which are costly to their offspring.

Methods

Study area and species

Lesser double-collared sunbirds were studied at Koeberg Nature Reserve (33° 41′S, 18° 27′E), South Africa. Between Aug. and Oct. 2005, 34 sunbird territories were regularly monitored for nest-building activity and egg laying. We caught 16 females originating form 16 different territories using mist-nets and measured tarsal length (to 0.1 mm) and body mass (to 0.01 g). Female body mass and tarsus length were normally distributed, and were unrelated to date of capture ($P = 0.29$ and $P = 0.84$, respectively, $n = 16$), or to each other ($P = 0.78$).

Clutch, egg and yolk measurements

We weighed 135 eggs from 62 clutches on 34 territories. Laying order and laying date were known for 101 eggs and most of those were weighed on the day of laying ($n = 75$) or the day after ($n = 19$). Six were measured on d two and one on d three. Some clutches were depredated before completion, and clutch size was known for 50 clutches (26 2-egg, 22 3-egg clutches and 2 single egg clutches).

Forty-two freshly laid eggs and nine one-day-old eggs of 28 first found clutches of 28 females were collected and stored at $-26°$ C until yolk androstenedione (A4) and testosterone (T) concentration could be analyzed. Homogenized yolks were mixed diluted 1:1 with water and extracted three times with 2 ml petroleum ether/diethylether (30/70%), followed by precipitation overnight with 70% methanol yielding recoveries of 82.1% or higher. Steroid concentrations were determined using highly specific antibodies for T and A4 respectively (cross reactivity for other hormones $<2\%$; I125 kits DSL, Webster) with an assay sensitivity of 0.03 ng/ml and 0.08 ng/ml for A4 and T, respectively. Intra-assay coefficients of variation were 8% for A4 and 4% for T. Hormone concentrations were normalized by log-transformation.

Statistical analyses

We used general linear mixed models (GLMM) in the program MLN 2.02 (Rasbash et al. 2000), which allows analyses of variance while taking into account the nested relationship of eggs within a clutch and clutches within females. Significance was tested using the change in deviance (Δdeviance, which follows a χ^2 distribution) when a factor was removed from the model (see text for details).

We used a stepwise backward modelling approach and ensured that no significantly auto-correlated parameters were entered into the same model. Parameters included in the models explaining yolk androgen concentration and egg mass variation in addition to laying order were egg age, laying date, female body mass, female tarsus length and clutch size. We first checked for potentially confounding effects of egg age and clutch initiation date, while female identity was included as a random term in the model. Egg age was included because some eggs were collected the day after laying: several studies (e.g. Elf and Fivizanni 2002, Eising et al. 2003b) have shown that yolk hormone concentrations can decrease substantially over the first 24 h. In addition, clutch initiation dates ranged over 52 d, which could have affected female reproductive decisions with respect to egg mass or yolk androgens. However, neither factor was significant in any model (all $P > 0.11$). We included female body mass and tarsal length because of the potential for an allometric relationship with egg mass (e.g. Saether 1987) and because studies of the relation between female body mass and yolk hormone levels have produced conflicting results (e.g. Pilz et al. 2003, Verboven et al. 2003). In this study however, we found no relation of female body mass with either egg mass or yolk T or A4 concentration (all $P > 0.28$) although there was an

effect on total yolk androgen concentration (see below). Finally, we included clutch size because the effects of hatching asynchrony were expected to be most pronounced in large clutches and a thus a more rapid increase in yolk hormones, egg mass or both could be expected over the laying sequence. However, no significant two-way interactions between clutch size and laying sequence were found for T and A4 concentrations or egg mass.

Results

Yolk hormones

Within eggs, Yolk T and A4 concentrations were not correlated ($r = 0.02$, $P = 0.87$), although T, A4 and total yolk androgen concentration did vary significantly between females ($\chi^2 = 25.50$, $P < 0.001$, $\chi^2 = 24.50$, $P < 0.001$, and $\chi^2 = 25.50$, $P < 0.001$, respectively). Factors of primary interest-laying order, clutch size and their interaction-were entered simultaneously into the model with female identity included as a random term. Models were then trimmed by sequential elimination of the least significant factor. This yielded no significant model for either T or A4 (Fig. 1a, b, all $P > 0.46$). However, total yolk androgen concentration (T+A4) did increase significantly with increasing female mass ($r = 0.27$, $\chi^2 = 5.36$, $P = 0.021$, $n = 7$).

Egg mass variation

Egg mass did not change significantly over the laying order (Fig. 1c; $\chi^2 = 0.04$, $P = 0.85$, $n = 106$). Likewise, there was no effect of clutch size ($\chi^2 = 0.32$, $P = 0.60$, $n = 113$) and no interaction effect between these predictors ($\chi^2 = 0.12$, $P = 0.73$, $n = 89$). Only the minimal model was significant ($\chi^2 = 67.49$, $P < 0.001$), containing the significant random effects of female identity ($\chi^2 = 8.91$, $P = 0.003$) and clutch identity ($\chi^2 = 50.97$, $P < 0.001$). Within females, there was a trend for egg mass to increase with sequential breeding attempts following clutch losses to predators ($\chi^2 = 3.44$, $P = 0.06$, $n = 135$).

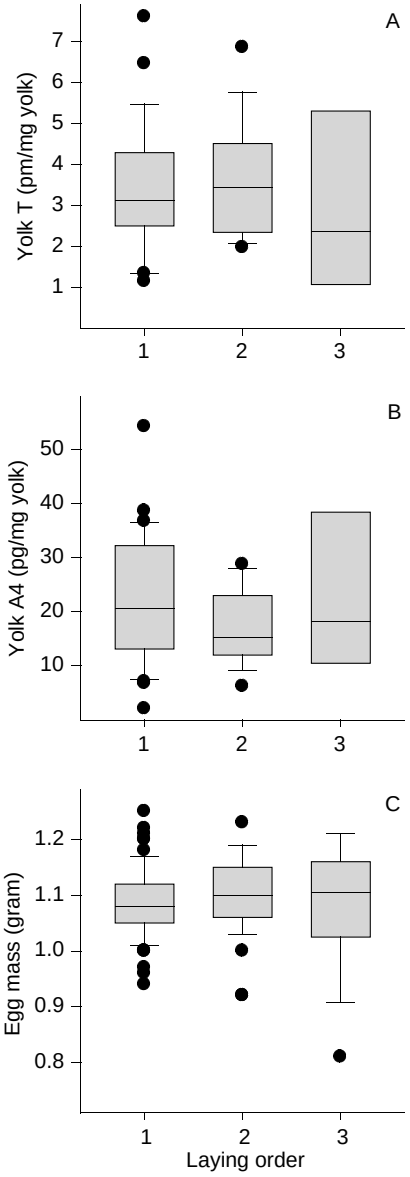


Figure 1. Yolk testosterone (A) and androstenedione (B) concentrations did not vary significantly across laying order in lesser double-collared sunbirds. Sample sizes for eggs of known laying order were 20, 18 and 5 for 1st-, 2nd- and 3rd-laid eggs respectively ($n = 25$ clutches). Egg mass (C) did also not increase over the laying sequence. Sample sizes for egg mass were 47, 43 and 16 for first-, second- and third-laid eggs respectively ($n = 50$ clutches of 27 females). Lower and upper boundaries of the boxes indicate the 25th and 75th percentile; error bars indicate the 10th and 90th percentile while the dots indicate outlying points.

Discussion

Although in lesser double-collared sunbirds the size hierarchy among chicks just after hatching is pronounced and still somewhat noticeable at 10 d of age (~5% weight difference; Schmidt 1964, Eising pers. obs.), this does not appear to translate into differential chick survival probabilities (see Introduction). This suggests that yolk androgens or egg mass compensate, at least partially, for these negative effects of hatching asynchrony. Contrary to these expectations, there was no increase of either yolk hormone concentration or egg mass across the laying order. These findings thus challenge the idea that, at least in this species, maternal yolk androgens play an important role in compensating for hatching asynchrony. Our results are consistent with those of Ellis et al. (2001), who also found: a) no significant increase in yolk hormone levels in asynchronously hatching clutches of house wrens, and b) that reproductive success was unaffected by the degree of hatching asynchrony.

Predictable or high food availability or quality can perhaps explain the lack of within-clutch variation in androgens as found in zebra finches *Taeniopygia guttata* (Sandell et al. 2007). Unfortunately, we have no data available on food availability. Therefore we cannot refute the compensation hypothesis as there could be within-clutch

differences in yolk androgen levels under adverse conditions.

In some asynchronously hatching species, where yolk hormone concentration does not vary predictably across the laying order, egg mass does (e.g. pied flycatchers *Ficedula hypoleuca*: Hillstrøm 1999, Tobler et al. 2007; collared flycatchers *Ficedula albicollis*: Hargitai et al. 2005, Michl et al. 2005). Because egg mass in general correlates with chick size, hatching from heavier last eggs (partially) compensates for the negative effects of HA (Rosivall et al. 2005). However, we found no repeatable pattern in egg mass variation within clutches at any stage during the breeding season. Rather, egg mass increased with each female's subsequent breeding attempt. This unusual finding (see Christians 2002) could be an adaptive response to the high rates of nest predation and the need, early in the season, to conserve energy for future breeding attempts. It seems unlikely that the cross-season increase in egg mass is simply a reflection of an equivalent increase in food availability as we found no effect of laying date per se or of temperature or rainfall (as proposed by e.g. Saino et al. 2004) in the week preceding an egg-laying event (all $P > 0.49$). Rather, this finding may reflect that late laying individuals opt for an 'all or nothing' strategy because chances of rearing a brood decrease as the breeding season progresses (e.g. Burger et al. 1996, Sanz 1998, Rossmanith et al. 2007).

The significant variation in egg mass between females could not be explained by either of the female characteristic measured here as proxies for body size although total yolk androgen concentration did increase with female body mass (also see Pilz et al. 2003). Potentially heavy females or their offspring are better able to withstand the costs that are associated with exposure to high yolk androgen levels (e.g. Groothuis et al. 2005b, Gil et al. 2006). Age or experience could also be important for determining maternal allocation (Christians 2002, Pilz et al. 2003) but we currently lack data to test this. Female reproductive investment may also reflect the quality of their breeding partner (see Groothuis et al. 2005a). Preliminary data in our study species suggest T and A4 concentrations may be related to two presumed male quality indicators - the size of the male's red breast-band ($B = 0.27$ and $B = 0.46$, respectively), and the number of feathers in the male's blue breast-band ($B = 0.03$ and $B = 0.06$, respectively) although we could not formally test this due to the small sample size ($n = 3$). Male parameters were not related to egg mass variation though.

In summary, differential maternal allocation in lesser double-collared sunbirds is more pronounced between females' clutches than within them. Currently, our data do not support the widely cited hypothesis that in a species with HA, differential maternal investment within clutches (e.g. in androgen levels) is necessary to compensate for negative effects on late-order hatchlings. We tentatively conclude that in this species detrimental effects of HA either do not occur or can be overcome in other ways. In general, such may depend on a species' life history or actual food availability. Further investigation is required to determine the role of yolk androgen variation, in combination with other investment strategies, in mitigating negative effects of hatching asynchrony.

Acknowledgements – This project was funded by a Claude Leon Foundation grant to C. Eising and DST/NRF Centre of Excellence funding to P. Lloyd. Many thanks to P. Lloyd and P. Hockey who provided valuable comments on an earlier draft. We thank B. de Vries for help with the hormone assay. Capture, banding and sampling activities were licensed by Cape Nature and SAFRING, the South African banding scheme (licence number 1059), and approved by the Anim. Ethics Comm., Univ. of Cape Town.

References

Boncoraglio, G., Rubolini, D., Romano, M., Martinelli, R. and Saino, N. 2006. Effects of elevated yolk androgens on perinatal begging behavior in yellow-legged gull (*Larus michahellis*) chicks. – Horm. Behav. 50: 442–447.

Burger, J., Nisbet, I. C. T, Safina, C. and Gochfeld, M. 1996. Temporal patterns in reproductive success in the endangered roseate tern (*Sterna dougallii*) nesting on Long Island, New York, and Bird Island, Massachusetts. – Auk 113: 131–142.

Clark, A. B. and Wilson, D. S. 1981. Avian breeding adaptations: hatching asynchrony, brood reduction and nest failure. – Q. Rev. Biol. 56: 253–277.

Cook, M. I., Beissinger, S. R., Toranzos, G. A., Rodriguez, R. A. and Arendt, W. J. 2003. Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? – Proc. R. Soc. B 270: 2233–2240.

Christians, J. K. 2002. Avian egg size: variation within species and inflexibility within individuals. – Biol. Rev. 77: 1–26.

Eising, C. M. and Groothuis, T. G. G. 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. – Anim. Behav. 66: 1027–1034.

Eising, C. M., Eikenaar, C., Schwabl, H. and Groothuis, T. G. G. 2001. Maternal androgens in black-headed gull eggs: consequences for chick development. – Proc. R. Soc. B 268: 839–846.

Eising, C. M., Müller, W., Dijkstra, C. and Groothuis, T. G. G. 2003. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. – Gen. Comp. Endocrinol. 132: 241–247.

Elf, P. K. and Fivizanni, A. J. 2002. Changes in sex steroid levels in yolks of the leghorn chicken, *Gallus domesticus*, during embryonic development. – J. Exp. Zool. 293: 594–600.

Ellis, L. A., Borst, D. W. and Thompson, C. F. 2001. Hatching asynchrony and maternal androgens in egg yolks of house wrens. – J. Avian Biol. 32: 26–30.

Forbes, S., Thornton, S., Glassey, B., Forbes, M. and Buckley N. J. 1997. Why parent birds play favourites. – Nature 6658: 351–352.

Gil, D., Biard, C., Lacroix, A., Spottiswoode, C. N., Saino, N., Puerta, M. and Møller, A. P. 2007. Evolution of yolk androgens in birds: development, coloniality, and sexual dichromatism. – Am. Nat. 169: 802–819.

Gil, D., Marzal, A., de Lope, F., Puerta, M. and Møller, A. P. 2006. Female house martins (*Delichon urbica*) reduce egg androgen deposition in response to a challenge of their immune system. – Behav. Ecol. Soc. 60: 96–100.

Gorman, K. B. and Williams, T. D. 2005. Correlated evolution of maternally derived yolk testosterone and early developmental traits in passerine birds. – Biol. Lett. 1: 461–464.

Groothuis, T. G. G., Müller, W., von Engelhardt, N., Carere, C. and Eising, C. M. 2005a. Maternal hormones as a tool to adjust offspring phenotype in avian species. – Neurosci. Biobehav. Rev. 29: 329–352.

Groothuis, T. G. G., Eising, C. M., Dijkstra, C. and Müller, W. 2005b. Balancing between costs and benefits of maternal hormone deposition in avian eggs. – Biol. Lett. 1: 78–81.
Hargitai, R., Török, J., Tőth, L., Hegyi, G., Rosivall, B., Szigeti, B. and Szöllősi, E. 2005. Effects of environmental conditions and parental quality on inter- and intraclutch egg-size variation in the collared flycatcher. – Auk 122: 509–522.
Hillstrøm, L. 1999. Variation in egg mass in the pied flycatcher: an experimental test of the brood survival and brood reduction hypotheses. – Evol. Ecol. Res. 1: 753–768.
Lack, D. 1947. The significance of clutch size. – Ibis 89: 302–352.
Lipar, J. L. 2001. Yolk steroids and the development of the hatching muscle in nestling European starlings. – J. Avian Biol. 32: 231–238.
Michl, G., Török, J., Péczely, P., Garamszegi, L. Z. and Schwabl, H. 2005. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. – Behav. Ecol. 16: 383–388.
Müller, W, Eising, C. M., Dijkstra, C. and Groothuis, T. G. G. 2004. Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed gulls. – Behav. Ecol. 15: 893–897.
Müller, W., Groothuis, T. G. G., Kasprzik, A., Dijkstra, C., Alatalo, R. V. and Siitari, H. 2005. Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks. – Proc. R. Soc. B 272: 1971–1977.
Pilz, K. M., Smith, H. G., Sandell, M. I. and Schabl, H. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? – Anim. Behav. 65: 841–850.
Pilz, K. M., Quiroga, M., Schwabl, H. and Adkins-Regan, E. 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. – Horm. Behav. 46: 179–192.
Rasbash J., Browne, W., Healy, M., Cameron, B. and Charlton, C. 2000. Multilevel models project. – Univ. of London, Press . . .?.
Richter, W. 1982. Hatching asynchrony-the nest failure hypothesis and brood reduction. – Am. Nat. 120: 828–832.
Rosivall, B., Szöllősi, E. and Török, J. 2005. Maternal compensation for hatching asynchrony in the collared flycatcher. – J. Avian Biol. 36: 531–537.
Rossmanith, E., Hontsch, K., Blaum, N. and Jeltsch, F. 2007. Reproductive success and nestling diet in the lesser spotted woodpecker (*Picoides minor*): the early bird gets the caterpillar. – J. Ornithol. 148: 323–332.
Saether, B. E. 1987. The influence of body weight on the covariation between reproductive traits in European birds. – Oikos 48: 79–88.
Saino, N., Romano, M., Ambrosini, R., Ferrari, R. P. and Møller, A. P. 2004. Timing of reproduction and egg quality covary with temperature in the insectivorous barn swallow. – Funct. Ecol. 18: 50–57.
Sandell, M. I., Adkins-Regan, E. and Ketterson, E. D. 2007. Pre-breeding diet affects the allocation of yolk hormones in zebra finches. – J. Avian Biol. 38: 284–290.
Sanz, J. J. 1998. Effects of geographic location and habitat on breeding parameters of great tits. – Auk 115: 1034–1051.
Schmidt, R. K. 1964. lesser double-collared sunbird *Cinnyris Chalybeus* in the south-western Cape. – Ostrich 35: 86–94.
Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. – Proc. Natl. Acad. Sci. USA 90: 11446–11450.
Schwabl, H., Palacios, M.G. and Martin, T. E. 2007. Selection for rapid embryo development correlates with embryo exposure to maternal androgens among passerine birds. – Am. Nat. 170: 196–206.
Sockman, K. W. and Schwabl, H. 2000. Yolk androgens reduce offspring survival. – Proc. R. Soc. B 267: 1451–1456.
Starck, J. M. and Ricklefs, R. E. (eds). 1998. Avian growth and development. – Oxford Univ. Press.
Stenning, M. J. 1996. Hatching asynchrony, brood reduction and other rapidly reproducing hypotheses. – Trends Ecol. Evol. 11: 243–246.
Skead, C. J. 1967. The sunbirds of southern Africa. – A. A. Balkema, Cape Town.
Tobler, M., Granbom, M. and Sandell, M. I. 2007. Maternal androgens in the pied flycatcher: timing of breeding and within-female consistency. – Oecologia 151: 731–740.
Veiga, J. P. 1992. Hatching asynchrony in the house sparrow-a test of the egg-viability hypothesis. – Am. Nat. 139: 669–675.
Verboven, N., Monaghan, P., Evans, D. M., Schwabl, H., Evans, N., Whitelaw, C. and Nager, R. G. 2003. Maternal condition, yolk androgens and offspring performance: a supplemental feeding experiment in the lesser black-backed gull. – Proc. R. Soc. B 270: 2223–2232.